

Three-chamber task

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 An abbreviated version of this protocol was published in eLIFE in Apr 2022

Oxytocin neurons mediate the effect of social isolation via the VTA circuits

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Detailed protocol

Three-chamber task

A three-chambered social interaction assay comprise a rectangular Plexiglas arena (60 × 40 × 22 cm with opaque walls) (Ugo Basile, Varese, Italy) divided into three chambers (each 20 × 40 × 22 (h) cm) separated by two doors. The experimental mouse is placed in the arena for a habituation period of 10 min when it is allowed to explore the empty arena freely. At the end of the habituation, the experimental mouse is gently accompanied in the central chamber, doors are closed and two enclosures with vertical metal bars are placed in the center of the two outer chambers. One enclosure is empty (serving as an object), whereas the other contains a social stimulus (stimulus1, 1week younger unfamiliar sex-matched conspecific, habituated to the enclosure for 20min a day, 3 days before the task). Doors are opened, and the experimental mouse is allowed to freely explore the apparatus and the enclosures for 10 min (social preference phase). After 10 minutes, the experimental mouse is again accompanied in the central chamber, and, after the closure of the doors, the empty enclosure is replaced with another containing an unfamiliar conspecific social stimulus (stimulus 2). Doors are opened, and the experimental mouse is allowed to freely explore for 10 min the apparatus and the enclosures (social novelty phase). The position of the empty vs. social stimulus1-containing or social stimulus1-containing vs. social stimulus 2-containing enclosures is alternated and is counterbalanced for each trial to avoid any bias effects. At the end of the test, all the mice (experimentals and stimuli) are returned in the home cages. Every session is video-tracked and recorded. The time spent interacting with each enclosure is manually scored and then used to determine the preference index for the object or social target (stimulus 1 and stimulus 2). The stimulus interaction is scored when the nose of the experimental mouse is oriented toward the enclosures at a distance approximately less than 2 cm. The arena is cleaned with 1% acetic acid solution and dried between trials. For the analysis, "Preference index" is calculated using the following formula: $\text{interaction time target 1} / (\text{interaction time target 1} + \text{interaction time target 2})$ or $\text{interaction time target 2} / (\text{interaction time target 1} + \text{interaction time target 2})$. Using this formula, the threshold is 0.5 which corresponds to the chance level to explore either target.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Musardo, S. and Bellone, C. (2022). Three-chamber task. Bio-protocol Preprint. bio-protocol.org/prep1850.
2. Musardo, S., Contestabile, A., Knoop, M., Baud, O. and Bellone, C. (2022). Oxytocin neurons mediate the effect of social isolation via the VTA circuits. eLIFE. DOI: [10.7554/eLife.73421](https://doi.org/10.7554/eLife.73421)

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